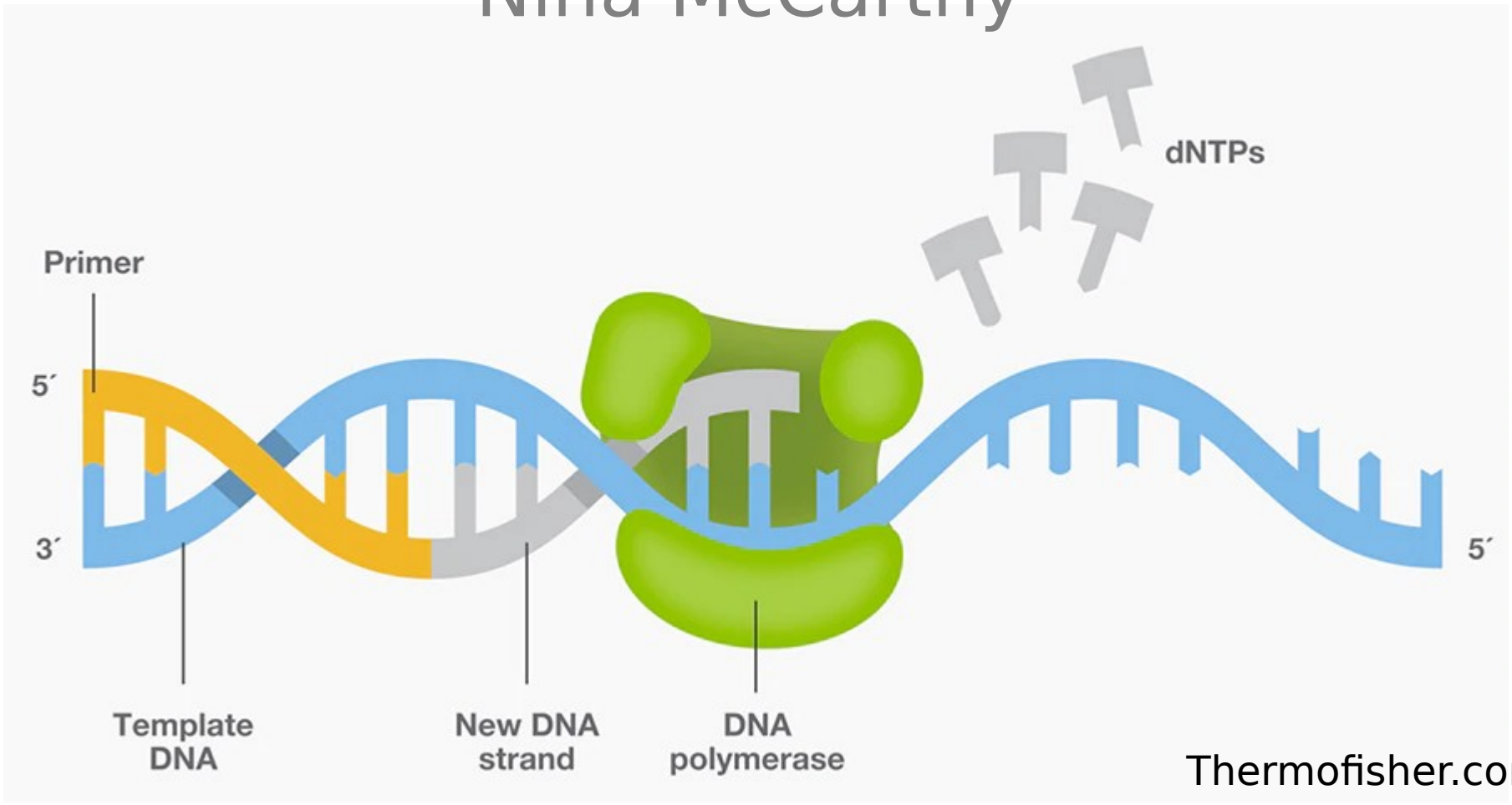
GENE2230 Laboratory Techniques

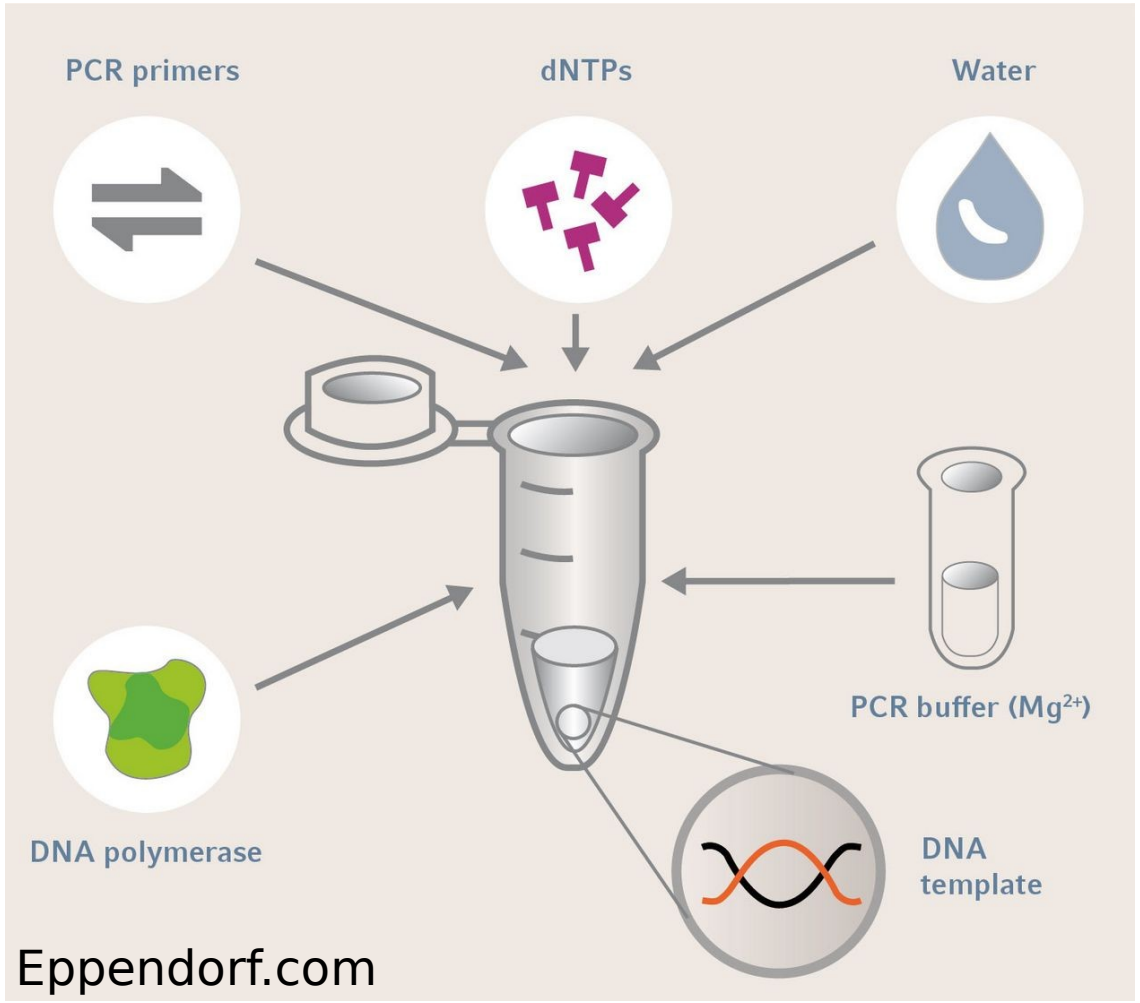
Nina McCarthy



Polymerase Chain Reaction (PCR) and Gel Electrophoresis

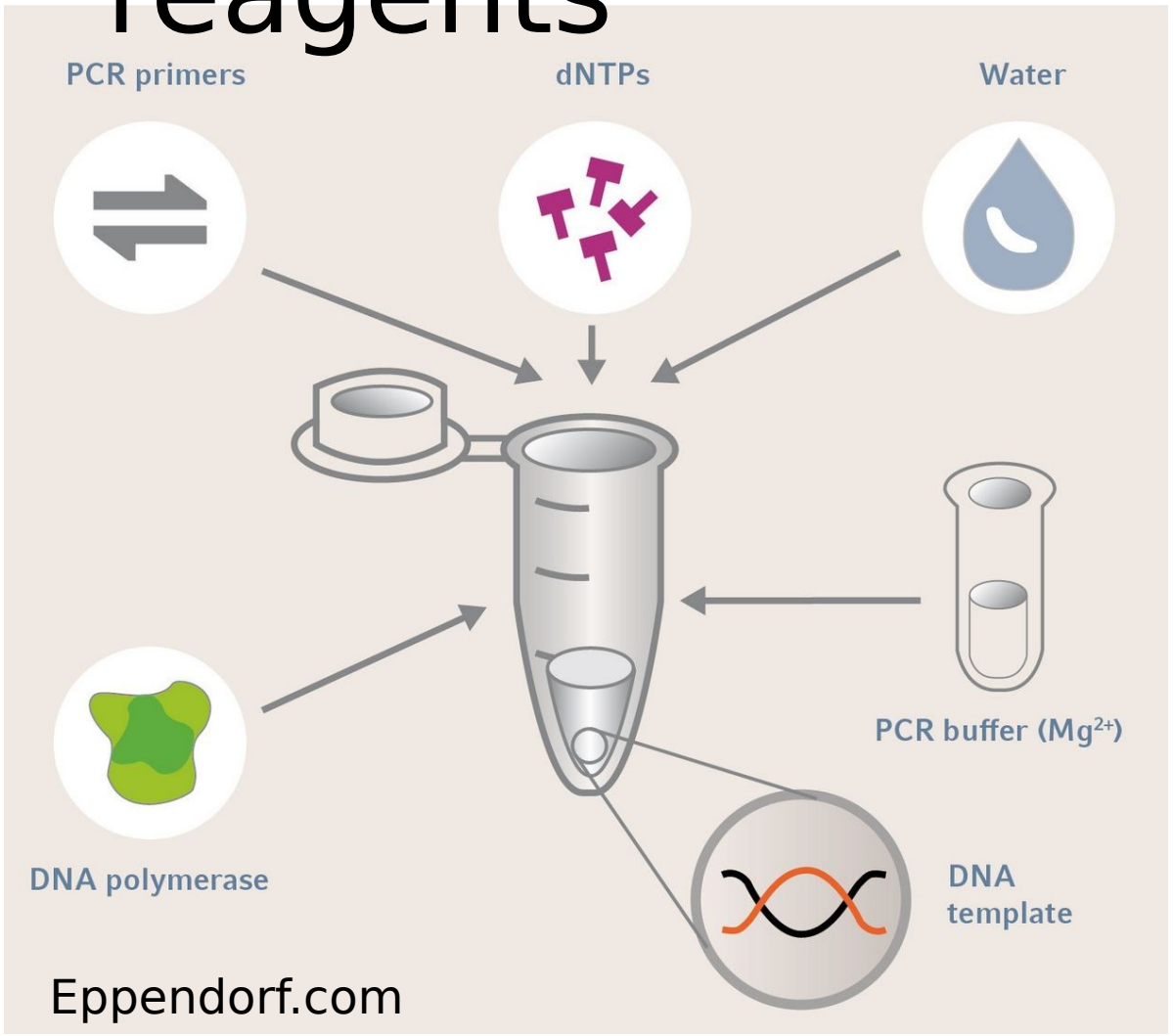
Polymerase chain reaction (PCR) is a technique to make many copies of a specific DNA region in vitro

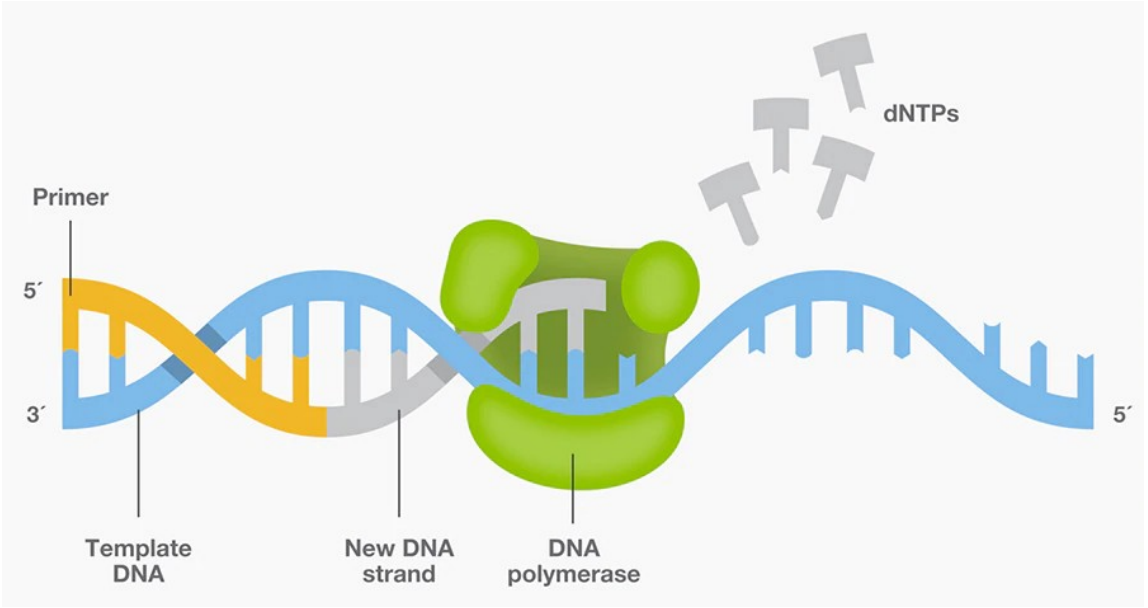
PCR reagents



Thermal cycler

PCR reagents

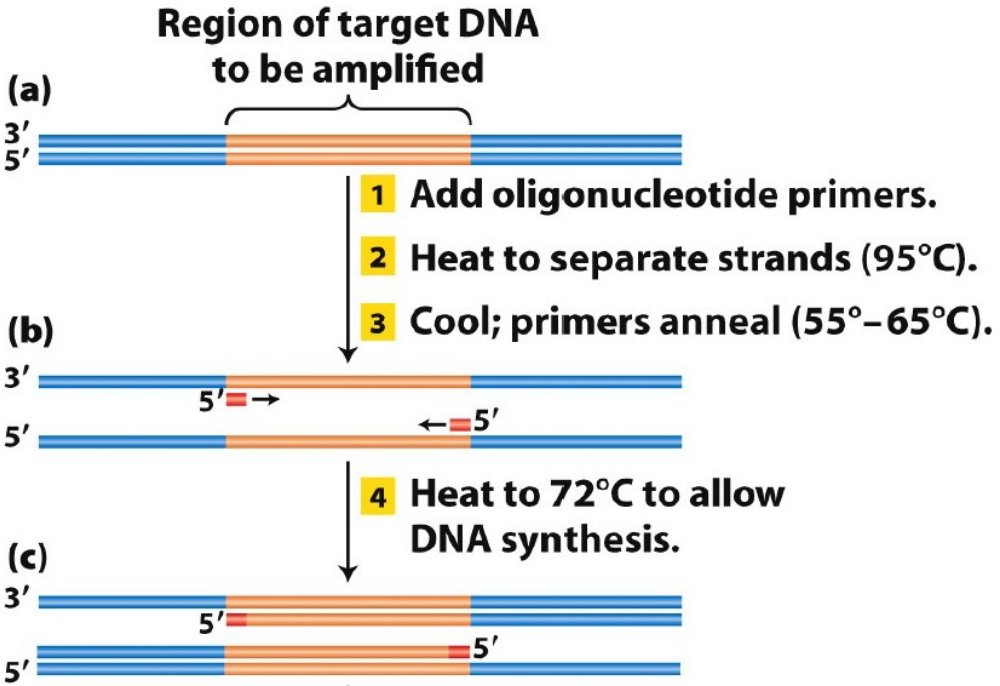




- Target/template DNA
- Two oligonucleotide primers
- A thermostable DNA polymerase (Taq)
- Deoxynucleotide triphosphates (dNTPs)
- Reaction buffer
- Magnesium (Mg^{2+}) - necessary for DNA polymerases to incorporate dNTPs - catalyzes formation of the phosphodiester bond between the 3´-OH of the new DNA strand and the phosphate group of a dNTP

Thermal cycler

- Cycling parameters are optimized for each template and primer pair combination.
- Annealing temperature: primer sequence is a major factor that determines the optimal annealing temperature, which is often within 5°C of the melting temperature (Tm) of the primers.
  - Annealing temperature lower than the primer Tm can result in higher yields, as the primers anneal more efficiently at the lower temperature. BUT can get nonspecific annealing and undesired products
  - Annealing temperature slightly higher than the primer Tm will increase annealing stringency (but lower yield)



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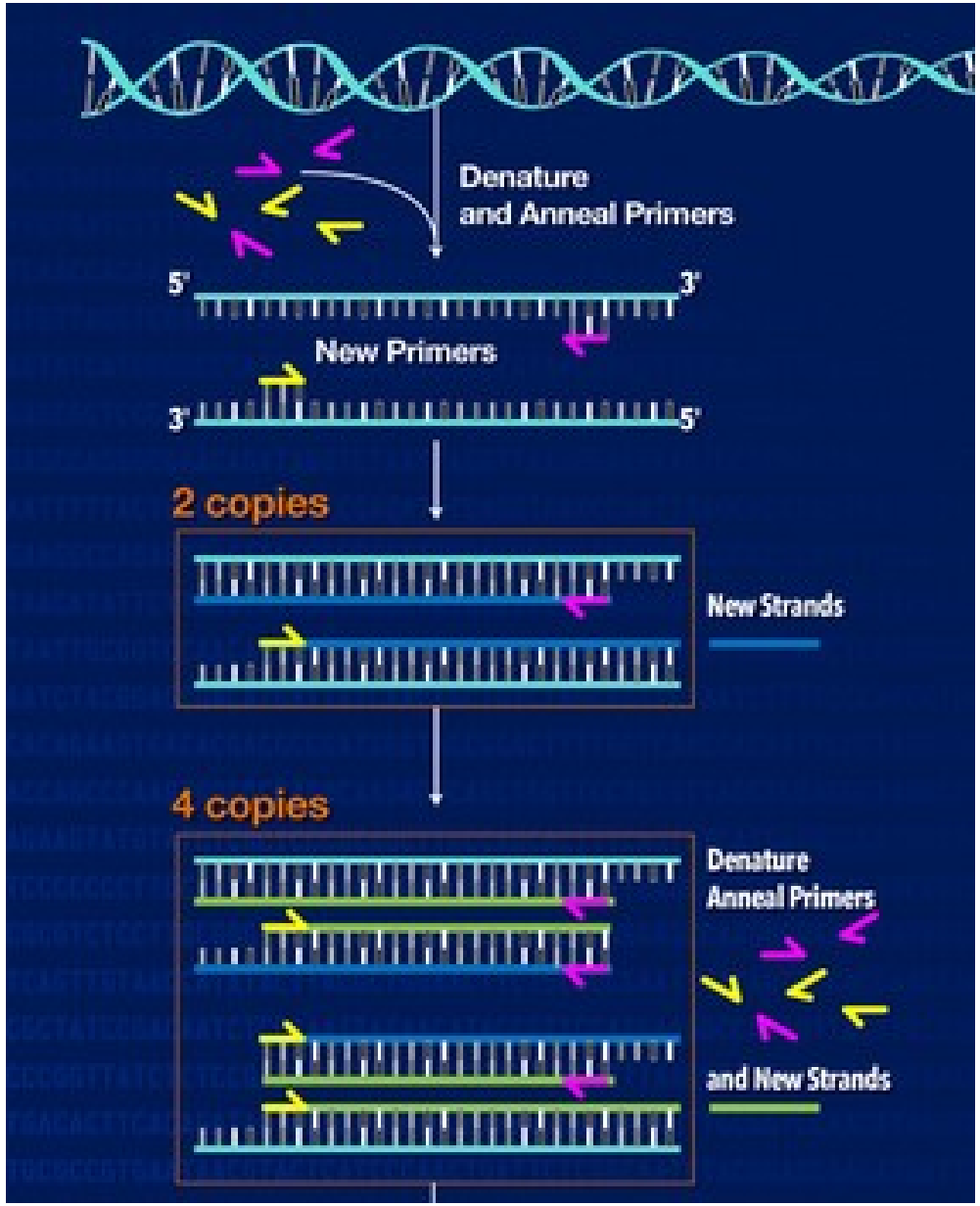
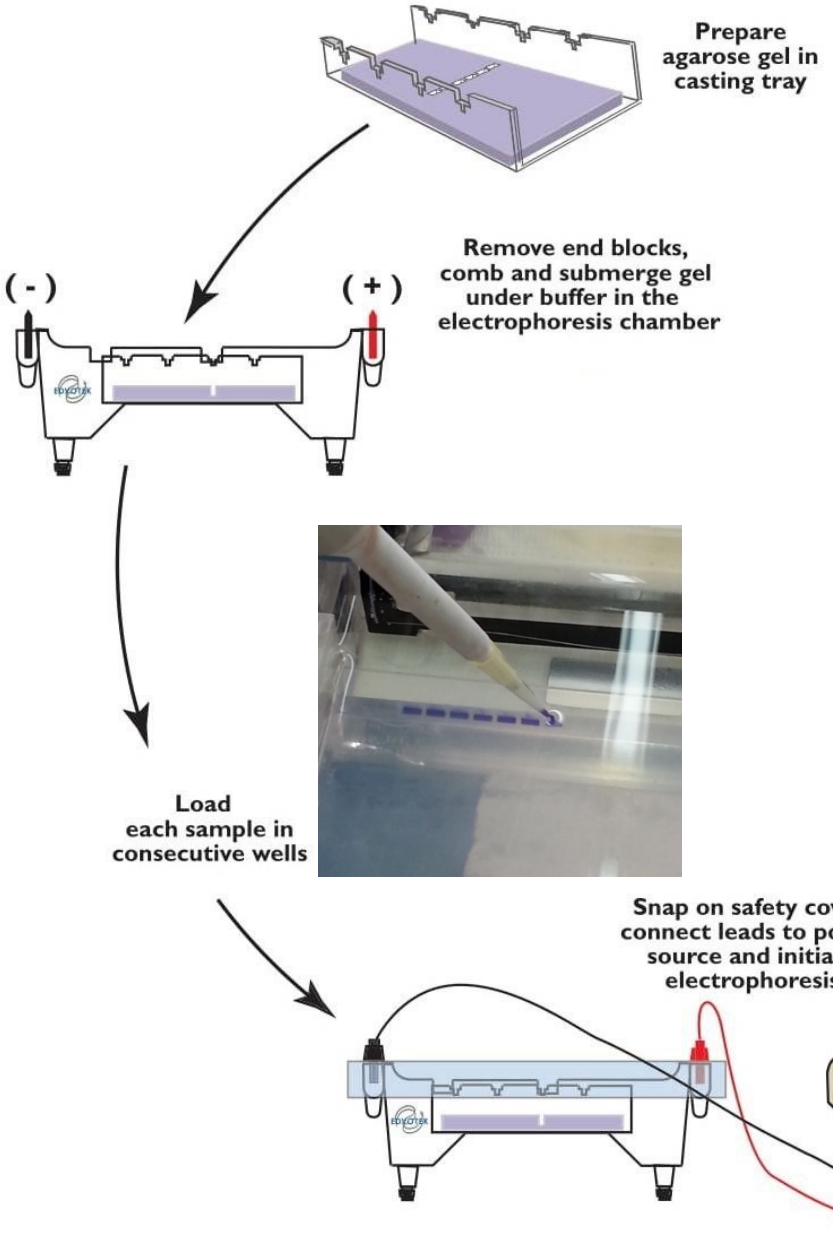


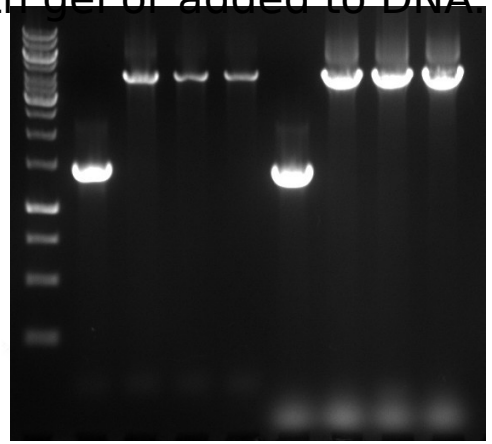
www.genome.gov

Agarose gel electrophoresis


Prepare agarose gel in casting tray
Remove end blocks, comb and submerge gel under buffer in the electrophoresis chamber
(-)
(+)
Load each sample in consecutive wells
Snap on safety cover, connect leads to power source and initiate electrophoresis

- Make gel: dissolve agarose in buffer – depending on size of DNA molecules, about 0.8% to 2% agarose gel used. Once set, gel is placed in an electrophoresis chamber and submerged in buffer.
- DNA sample (plus ladder containing known lengths of DNA for comparison) mixed with loading buffer (dense compound + dye) and sample loaded.
- Electric field applied (5-10V per cm) – negatively charged DNA molecules migrate towards the positively charged electrode. Gel matrix creates resistance – smaller molecules migrate more quickly, larger molecules migrate more slowly (DNA molecules separated according to their length).
- Visualise using UV light - mix with GelRed/ethidium bromide (intercalating agent, fluoresces under UV light. Potential carcinogen). Can be mixed in with gel or added to DNA.

Visualise gel in a gel doc



Adapted by NMc from https://microbeonline.com/agarose-gel-

Resources

- PCR basics: https://www.khanacademy.org/science/ap-biology/gene-expression-and-regulation/biotechnology/v/the-polymerase-chain-reaction-pcr
- PCR details:https://www.promega.com.au/resources/guides/nucleic-acid-analysis/pcr-amplification/#general-considerations-for-pcr-optimization-6f575242-fe99-4fcb-88c5-b205ad7becc7
- PCR factsheet: https://www.genome.gov/about-genomics/fact-sheets/Polymerase-Chain-Reaction-Fact-Sheet
- Agarose gel electrophoresis video: https://www.youtube.com/watch?v=vq759wKCCUQ
- Agarose gel basics: https://www.youtube.com/watch?v=MhJT9yjnl88
- Gel electrophoresis: https://www.khanacademy.org/science/ap-biology/gene-expression-and-regulation/biotechnology/v/gel-electrophoresis-dna